JOURNAL OF FUNCTIONAL FOODS 5 (2013) 2024–2029

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Short communications

Elucidation of structural difference in theaflavins for modulation of starch digestion

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ARTICLE INFO

Article history:
Received 3 August 2013
Received in revised form
9 September 2013
Accepted 25 September 2013
Available online 16 October 2013

Keywords:
Theaflavins
Inhibition
Structure
Starch digestion
Human α-Amylase

ABSTRACT

The relationship between structure and activity of theaflavins against human pancreatic α-Amylase was investigated by *in vitro* and *in silico* methods. The IC_{50} and total energy value showed that inhibitory effects followed the order: theaflavin-3, 3'-di-*O*-gallate > theaflavin-3'-*O*-gallate > theaflavin-3-*O*-gallate > theaflavin. Inhibitory activity was depended on hydroxyl groups and galloyl moieties of theaflavins to interact with the catalytic residues of the active site of α-Amylase by hydrogen bonds and π–π (aromatic–aromatic) interactions. The galloylated theaflavin has higher binding affinity with α-Amylase than non-galloylated theaflavin. The study showed that theaflavins might act as natural enzyme inhibitors with potential health benefits, which provide a foundation for designing novel functional food for effective controlling of starch digestion and postprandial glucose levels.

1. Introduction

Starch is the main glycemic carbohydrate in cereal products and the glucose generated from starch digestion plays an important role in energy metabolism and glucose homeostasis (Apostolidis, Li, Lee, & Seeram, 2011; Aston, 2006; Manuel-y-Keenoy & Perez-Gallardo, 2012; Marshall, 2006; Zhang & Hamaker, 2009). Poorly modulation of starch digestion is associated with an increased risk of developing diabetes, prediabetes, cardiovascular disease, and obesity (Englyst & Englyst, 2005; Ludwig, 2002). In the past decades, considerable research effort has been devoted to novel ways for effective controlling of blood glucose and prevention of related diseases (Judy et al., 2010; Miao, Zhang, Mu, & Jiang, 2010; Zhang & Hamaker, 2009). Compared to the prescription medicine (i.e. Acarbose and Phase II), novel functional foods and beverages for improvement of postprandial hyperglycemia have become an attractive alternative in slowing down glucose production from dietary carbohydrate and absorption with fewer gastrointestinal side effects (Englyst & Englyst, 2005; Judy et al., 2010; McClements, Decker, Park, & Weiss, 2009; Miao et al., 2010; Miao et al., 2014).

Tea is one of the most popular beverages consumed worldwide and has been proved to be capable of preventing various chronic diseases, such as cancer, inflammation, osteoporosis, cardiovascular diseases and obesity, which is largely contributed by its rich flavonoids and polyphenols (Chandrasekara & Shahidi, 2012; Ho, Lin, & Shahidi, 2008; Huang, Liu, Dushen-

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1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.09.021

kov, Ho, & Huang, 2009; Tsai, Hsu, Ting, Huang, & Yen, 2013). However, there is little study to data that has examined the theaflavins for modulation of starch digestion. The objective of experiment was to study the interactions between human α-Amylase and individual theaflavins with *in vitro* and *in silico* methods, giving insight into the structural requirements and molecular mechanism for action of enzyme inhibitor.

2. Materials and methods

2.1. Materials

Theaflavin (Cat. No. 201-15161), theaflavin-3-O-gallate (Cat. No. 202-15191), theaflavin-3'-O-gallate (Cat. No. 204-15271) and theaflavin-3, 3'-di-O-gallate (Cat. No. 208-15171) from black tea were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acarbose (Cat. No. A-8980, ⩾95%) and human pancreatic α-Amylase (Cat. No. A-9972, ⩾100 units/mg protein) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Normal maize starch was obtained from Changchun Dacheng Industrial Group Co. (Jilin, China). All chemicals were reagent grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Alpha-Amylase assays

Enzyme solution was prepared by suspending human α-Amylase (0.2 g) in phosphate buffer (6 ml, 0.2 M, pH 5.2) with magnetic stirring for 10 min, centrifuging the mixture for 10 min at 1500 × g, then transferring a portion (5 ml) of the supernatant into a beaker. Theaflavins or acarbose was prepared in phosphate buffer (0.2 M, pH 5.2) and diluted into a range of desired concentration (0, 0.1 μM, 1 μM, 10 μM, 100 μM, 1 mM, 10 mM and 100 mM). The maize starch sample (20 mg) was dissolved in 10 ml of phosphate buffer (0.2 M, pH 5.2) by heating at 95 °C for 10 min. After the solution was cooled and equilibrated at 37 °C for 5 min, sample of inhibitor (75 μl) and enzyme solution (15 μl) were added to 10 μl of starch solution. Then, the reactions were carried out in a 37 °C water bath for 15 min. The reducing sugar content was determined with the Nelson-Somogyi procedure by measuring the absorbance at 540 nm. A control vial was prepared by replacing the inhibitor solution with phosphate buffer. IC_{50} value (half maximal inhibitory concentration) was obtained graphically by an inhibition curve. All analyses were performed in duplicate.

2.3. Docking studies

The three-dimensional structure of human pancreatic α-Amylase was imported from the Protein Data Bank (1HNY). The structure of theaflavins was generated with the Cambridge Soft ChemBioDraw Ultra (Version 12.0) and energy minimized with the MM2 calculations using a conjugate gradient. Before the docking procedure, water molecules were removed from the enzyme crytal structure using Accelrys Discovery Studio 3.0 software. Automated molecular docking study of the inhibitory ligand at the amylase-binding site was performed with the AutoDock 4.2 package. The evaluation procedure of the molecular docking was performed according to the scores of several scoring functions. According to the scores and binding-energy value, the best pose for theaflavins was obtained. Hydrogen bonds and Van der Waals forces interactions were also obtained from the docking results.

3. Results and discussion

3.1. Inhibition of human α-Amylase activity

Inhibitory potency of individual theaflavins against human pancreatic α-Amylase is determined, and the IC_{50} is summarized in Table 1. The results of IC_{50} showed that inhibitory effects followed the order: theaflavin-3, 3'-di-O-gallate > acarbose > theaflavin-3'-O-gallate > theaflavin-3-O-gallate > theaflavin, which indicated that the inhibitory potency of theaflavin-3, 3'-di-O-gallate ranked the first having the IC_{50} of 2.6 μM and was more than 10-fold than that of theaflavin (IC_{50} = 33.7 μM) using therapeutic drug acarbose as a benchmark. Similar observations for inhibitory trend of tea polyphenols have been reported by Hara and Honda (1990) and Koh, Wong, Loo, Kasapis, and Huang (2010). It is known to all that acarbose is an anti-diabetic drug used to treat type 2 diabetes mellitus and prediabetes. Acarbose inhibits enzymes needed to digest starch in the small intestine, which will be delivered to the colon and cause gastrointestinal side-effects. Therefore, natural molecules with potential health benefits have become a more acceptable source of bioactive agents for slowing down glucose production. Theaflavins are characteristic antioxidant polyphenols in black tea that are formed from flavanols during the enzymatic oxidation (fully fermented) (Ho et al., 2008). As shown in Fig. 1 (A), the theaflavins shared a common structural scaffold consisting of a planar benzotropolone ring system, with two flavan rings approximately perpendicular to this plane, and stacked against each other. From the results, it could also be deduced that the number of galloyl (3, 4, 5-trihydroxybenzoyl) and substituted positions of flavan rings (R3 or R3' in the A–C condensate ring system) were critical in determining inhibitory activity. According to a review of Bandyopadhyay, Ghosh, and Ghosh (2012), polyphenols interacted with α-Amylase primarily through non-covalent interactions (hydrogen bonding

Table 1 – Summary of inhibiting and docking parameters of theaflavins against human α-Amylase.

Inhibitor	IC_{50} (μM)	Total energy (kJ/mol)	Hydrogen bonds energy (kJ/mol)	Van der Waals energy (kJ/mol)
Theaflavin	33.7	−119.69	−38.72	−80.98
Theaflavin-3-O-gallate	5.1	−141.75	−35.63	−106.11
Theaflavin-3'-O-gallate	4.0	−159.30	−41.85	−117.45
Theaflavin-3,3'-di-O-gallate	2.6	−167.94	−64.20	−103.74
Acarbose	3.8	−143.20	−42.36	−100.80